rchives

ta \bī-'ōt-ə\ *n* [NL, fr. Gk *biotē*; akin
Gk *bios*]: the flora and fauna of a
ion

io·tech·nol·o·gy \bī(,)ō-tek-'näl-ə-jē\ *n*
1: the aspect of technology concerned
with the application of biological and
engineering data to problems relating to
plants, humans and animals **2:** the use
of living organisms or their components
in industrial processes **3:** improving or
modifying organisms, microbes, plants or
animals, and new research techniques or
technology **4:** refers to contemporary
tools available to scientists for purposes
of biotechnology development

io·tech·nol·o·gist \bī(,)ō-tek-'näl-ə-jəst\
n: a scientist who deals in the field of
biotechnology

i·ot·ic \bī-'ät-ik\ *adj* [Gk *biōtikos*, fr
bioun]: of or relating to life; *esp*: caused
by living beings

ANNUAL REPORT

i·o **ic** \(1) bī-'ä-tik\ *adj* ob
fr. NL *bioticus*, fr. Gk *biotikos*]: having
a (specified) mode of life ‹endo*biotic*›

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bio·tech·nol·o·gy

...studying and using living organisms or their components to improve plants and animals... is not really a new concept in agricultural research. Our ancestors 10,000 years ago engaged in a primitive form of biotechnology to improve the crops they harvested and the animals they domesticated. For at least the last 80 years, agricultural researchers have been using developing forms of this technology. In the last 20 years, science's newfound understanding of basic biological systems accelerated our progress in biotechnology.

Today, this understanding has helped immunologists develop methods of animal disease detection so rapid and precise that had these methods been available just 2 years ago, they might have saved the mid-Atlantic poultry industry millions of dollars. Using this knowledge of plants and animals at the molecular level, pathologists have been able to characterize chemical compounds heretofore unknown; compounds that promise to be the future for environmentally sound pest controls. Advances in biotechnology promise to shed light on basic triggering mechanisms responsible for life developing from seemingly undifferentiated clumps of tissue.

Applying biotechnology to solve important agricultural problems will require a combined effort, with researchers from microbiology, immunology and biochemistry, as well as the traditional agricultural disciplines. Integrating the basic sciences with applied agricultural sciences requires a coordination of programs. By its very nature and structure, the land grant university is the ideal setting for such an endeavor.

However, we have not remained isolated. In May 1984, The University of Maryland signed a new agreement with the U.S. Department of Agriculture to collaborate on studies in biotechnology and to pool scarce resources toward that collaborative goal. We have had a close working relationship with USDA's Agricultural Research Service since 1957. But this new agreement is the beginning of a pioneering relationship as we move together toward identical goals, into a new scientific frontier.

We invite you to examine this annual report of research efforts in biotechnology. We hope to bring you information on other studies that are not in this year's annual report in subsequent issues.

Let us hear your comments on our work,
and the way we have presented it to you.

W. LAMAR HARRIS • DIRECTOR • UMAES W Lamar Harris

Manipulating Plant Cells

* * *

Characterizing Their Components

Farmers have been cultivating plants for several thousand years. But over the years, the tools and techniques have changed significantly. Today there is a good chance that the food and fiber we will be eating will come from drastically altered plants.

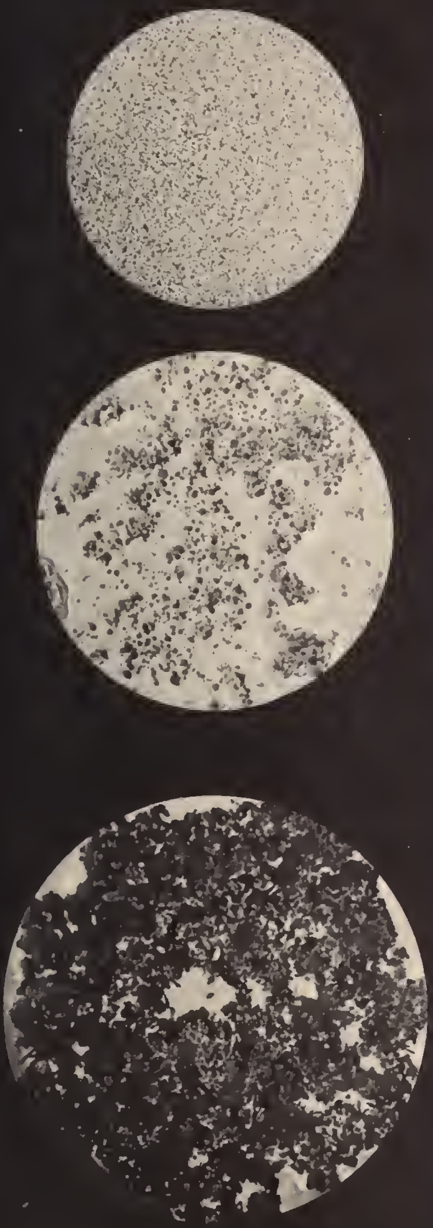
Can we combine the best genetic material of two related crop species, or two unrelated species to create custom-made plants? Can we make crops resistant to certain toxic substances, such as herbicides or heavy metals? Can resistance to disease be introduced into the genetic material of valuable crop plants? How does life spring from an undifferentiated mass of tissue into anything resembling a plant? What subcellular mechanism triggers the whole operation into action?

These are the kinds of questions biotechnologists in the plant sciences seek to answer. In spite of recent advances, plant biotechnologists are hampered by limited knowledge of the biology of plants. As one example of the mind-boggling complexity of plant biology, molecular biologists must identify agriculturally important genes from a pool of more than 5 million genes in most plants.

The following pieces illustrate some of the problems facing those working in biotechnology for UMAES, how they are dealing with those problems, and what the long range payoffs might be.

Biomembranes:

Nature's Gatekeepers Play a Major Role in the Future of Plant Breeding



Nature's gatekeepers, the **cellular membranes**, are microscopic in size and only one or two molecules thick. They are fluid, film-like buffers between what is important inside the cell, and what is potentially friendly or hostile outside.

Like any good gatekeeper, cellular membranes do more than organize cells and cell parts. They decide when, how, and how much material will be allowed in and out of cells, and between cells, according to botanist Dr. Paul J. Bottino.

"That basically means they command the chemistry and physics of living cells," says Bottino. "If it weren't for that, organisms such as plants would be unable to take in nutrition, grow, reproduce or eliminate waste products."

Scientists are spending a lot of time these days studying cellular membranes. Understanding how they work could provide the basis for advances in genetic engineering and biotechnology.

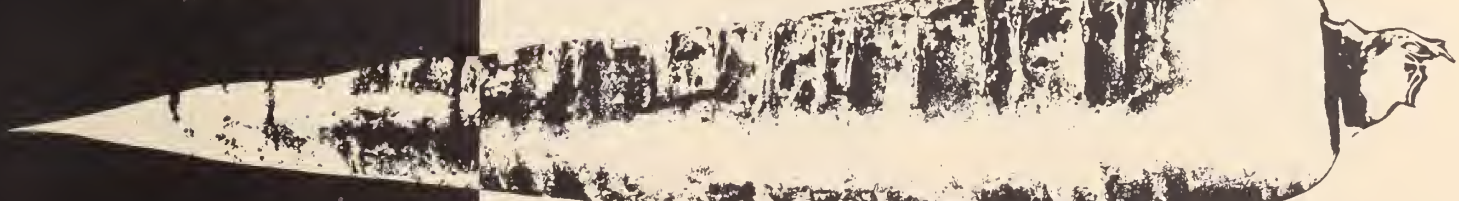
One of those advances is taking shape in the form of a technique called **protoplast fusion.** If you dig just beneath a plant cell's wall, you will encounter first the cell's membrane. And just beneath that is a "sac" — the **protoplast** — which holds the interior working parts of the cell. Fuse the protoplasts of two cells together and, theoretically, they become one, sharing their interior working parts and their genetic material. Protoplast fusion has the potential for creating new types of crop plant hybrids — genetically superior plants that are impossible to create using traditional breeding methods.

Future promise, however, is slow to translate into present reality. "In spite of all the work invested in protoplast fusion, no useful hybrid crop plant has yet come out of the process," says Bottino. He says that the key to a breakthrough may lie in learning more about those cellular membranes, using protoplast fusion studies as a springboard.

"Because protoplast fusion involves dissolving and reforming the plasma membrane, the process provides an excellent opportunity to study membrane interactions which occur during and following fusion," Bottino says.

One of the interactions between membranes that scientists have documented during the fusion process is **agglutination,** the process by which membrane surfaces "glue" themselves to each other during fusion.

A major interaction documented by scientists after the fusion process is **membrane redistribution,** or how the parts of two different cellular membranes reform after they have fused together. Generally, says Bottino, cell membrane surfaces are fluid enough to allow free intermixing of membrane components after fusion.



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Tissue Culture and New Varieties Could Put Eastern Shore Back on the Strawberry Throne!

After their overthrow by corn and soybeans, vegetables and small fruits, the strawberries may be ready to return from exile to Maryland's Eastern Shore.

Until recently, the strawberry nursery industry on the Eastern Shore has been stagnant, according to UMAES fruit breeder and assistant professor of horticulture Dr. Harry Jan Swartz.

A combination of factors lead to a period of rapid growth in strawberry culture on the Eastern Shore. The success story is being written by a combination of new biotechnologies like tissue culture, a long-term effort in strawberry breeding, the state's extraordinary climate and the strong horticultural background of Eastern Shore growers.

"Our recent research on the effects of tissue culture on strawberry nursery plant performance indicates to us that we could help commercial nurseries double their productivity."

Tissue culture is one of the new biotechnological tools scientists are using to improve varieties of fruits and vegetables. It is primarily a breeder's tool to expedite mass propagation of a single plant carrying desired characteristics, such as disease resistance or fruit quality. Theoretically, a tiny bit of plant tissue taken from a superior "parent" plant will grow into a mass of plant shoots in a culture medium. According to scientists, it is possible to regenerate from that mass of shoots hundreds or thousands of whole plants, each carrying the desired characteristics of the original donor plant.

When it comes to strawberries, Swartz says he has discovered a few other attributes of tissue culture. Not only are the products of strawberry tissue culture completely uniform — a very desirable attribute as far as the nursery industry is concerned — but their plants also produce more runners than the products of standard propagation methods.

"Even in overcrowded situations, tissue culture-propagated plants produced 50 percent more runners than standard-propagated plants," says Swartz. This, he adds, can contribute to a "too much of a good thing" situation. Nursery growers must exercise caution and limit their use of tissue culture to plant varieties that will not become too crowded for overall plant quality.

"In more recent work we've done, where we made sure the plants were not crowded, we had a sevenfold increase in runner production in tissue culture-propagated plants over standard plants," he says.

Some very practical information for nursery growers came out of those studies, too.

"Tissue culture plants produce more runners, but their ability to do so is only temporary," says Swartz. "Therefore, growers should move tissue culture stock into the field as soon as possible, within 4 months."

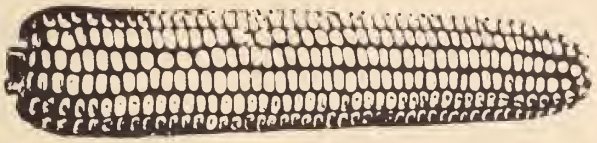
In the future, however, this phenomenon will allow changes in cultural systems in the east. This temporary increase in runner production will allow sufficient propagation of shy runnering everbearing varieties that are desired by the grower for newer, more productive systems of strawberry culture.

Swartz and his graduate students are using tissue culture in the Maryland strawberry and raspberry breeding program. "Tissue culture allows us to control more accurately the environment to which plants are exposed. Thus, experimentation on disease, pest or herbicide resistance, is enhanced."

Tissue culture can also be used to generate new types of plants in culture. This totally bypasses standard hybridization procedures and allows for production of heretofore impossible plant types. Graduate students in the program successfully developed plantlet regeneration, an important first step to genetic engineering for raspberries and strawberries.

The estimated payoff of a high-technology strawberry industry is approximately $40 million annually in sales to big city markets such as Philadelphia, Baltimore and Washington, D.C., and the Virginia tidewater.

The Biochemical Basis for Disease Resistance



What makes one kind of corn plant more resistant to disease than another? The answer, according to biochemists, lies in the genes. Molecular geneticists have known for some time that the DNA material in the mitochondria of disease-resistant corn differs from that in corn susceptible to such diseases as corn blight. Mitochondria are the energy-producing organelles found within the walls of corn and other living cells.

But how mitochondrial DNA differ and how that difference translates into the genetic basis for disease resistance is something scientists still do not fully understand.

"If we can determine what and how genetic material confers resistance in a plant, it should help us understand and control plant diseases," says biochemist Dr. Heven Sze, an assistant professor of botany. "It also could help geneticists develop genetically superior varieties of agricultural crops."

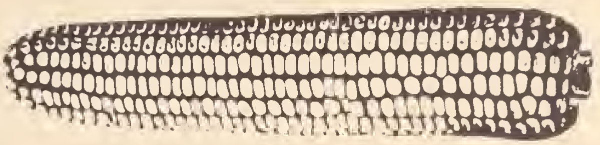
Gaining a better understanding of the genetic basis of disease resistance is precisely what Sze has been working on for UMAES since the fall of 1983. She chose corn as a plant model to study because its genetics are well understood by scientists. And, she chose **HmT toxin** (*Helminthosporium maydis* race T), as her model disease. HmT toxin is the active chemical in southern corn leaf blight, a scourge that seriously threatened hybrid corn and American agriculture in 1970 and 1971.

So far, Sze has found that the mitochondria are primary sites for the toxin to do its dirty work.

"The toxin appears to alter the normal function of cell membranes and the membranes of organelles, especially the mitochondria," says Sze.

In susceptible plants, the toxin makes it easier for certain kinds of ions — chemically charged particles such as calcium and hydrogen — to permeate the membranes of mitochondria.

"It basically opens a pore or channel for calcium ions, and probably hydrogen ions, to travel into the mitochondria," says Sze.



In plants resistant to the toxin, however, the membranes of mitochondria are not so permeable.

Scientists still need to find out the biological basis for resistance to the toxin in some types of mitochrondria and susceptibility in others.

"There are a number of questions about the difference between resistant and susceptible types we'd like to answer," says Sze. "For example, do susceptible mitochondria facilitate the opening of pores for the ions to enter, or is it that resistant mitochrondria make it more difficult?"

How important is this basic research to agriculture? According to her colleague, Dr. Jane F. Rissler, "What we know about pathogen gene products is rare enough. Even rarer, however, is information on how they work in host plants such as corn. If Dr. Sze determines completely how HmT toxin works in susceptible and resistant plants, it will be an important step toward our understanding of how plant structures and disease-causing molecules interact."

Engineering Tomorrow's Dinner

For the last 25 years, genetic engineers have spent most of their time worrying about the mechanics of recombining bits of DNA — the genetic messengers in plants and animals. The nuts and bolts of recombinant DNA technology may not present insurmountable problems now. But scientists have yet to figure out how to regenerate more than a few simple species of plants from engineered tissue into anything resembling a mature plant.

"The initial products of genetic manipulation are single, transformed cells — and not whole organisms," says Dr. Todd J. Cooke, assistant professor of botany.

There have been some successes. In the late 1950's scientists figured out how to transform engineered carrot and tobacco cells into entire plants, using hormones and growth regulators to prod the cells into embryonic development or **embryogenesis.**

But nutrition-packed crops such as legumes and cereals have proven more cantankerous, says Cooke's colleague, Dr. Richard H. Racusen.

Cooke and Racusen are working together on a theory that gained them a $110,000 USDA grant to find the basic mechanism that triggers embryogenesis.

"We think that, somehow, plant cells generate their own electrical fields, and that those fields govern what we know is the sequence of events we call embryogenesis," says Cooke.

In the first stages of embryogenesis, according to Cooke and Racusen, the interior parts of plant cells — called **organelles** — arrange themselves in a polarized fashion, sort of like a bar magnet. The continued growth of cells in a specific fashion — to form roots, stem and leaves — is influenced by that polarity phenomenon.

Racusen, who has done extensive work with cellular polarity since 1976, says each cell produces its own electrical field, which comes from the traffic of charged particles, such as hydrogen ions, across cell membranes.

Initially, Cooke and Racusen planned to prove or disprove their theory by figuring out how easily manipulated crop species such as carrots set up cellular electrical fields. They believe that proteins within the cell walls pump potassium, hydrogen and calcium ions across cell membranes. The pumping action sets up electrical fields and triggers embryogenesis, according to the theory. How plant genes are involved in the expression of ion pumping activity is a major part of the puzzle to be solved, they say.

Once that work is completed, Cooke and Racusen will move to a second stage of their project. They will try to manipulate the magnitude and direction of the electrical fields in cells, to get them to line up in the direction that leads to embryogenesis.

What is their most ambitious objective? "We want to use what we've learned about the process from the carrot experiments to induce embryogenesis in a more stubborn species, the soybean," says Cooke. "If it works, it could have important implications for genetic manipulation of all crop species."

Wheat Anther Culture:
One Way to Improve Tomorrow's Loaf

Anther culture, the process by which male sex organs in plants are prodded to produce adult plants, may help wheat researchers develop new varieties of wheat that promise increased yields and survival in a range of environments.

"If we can find out how to improve the frequencies of anther culture response, it will significantly reduce the labor required to produce some lines of crop plants," say agronomists Joy Marburger and David Sammons.

Anthers, the male sex organs of plants, normally produce pollen cells that carry sperm to fertilize the plant's egg cells. And in normal plant development, the end product of this fertilization process is seeds.

But pollen can be guided into another developmental pathway, using anther culture, according to Marburger and Sammons. Immature anthers are removed from the plant and cultured on a nutrient medium in test tubes. Instead of forming sex cells or sperm, the pollen produced by the cultured anthers develops into a mass of cream colored tissue called **callus.**

"In some crops, like tobacco, small plants develop from these pollen cells," says Marburger. "But with wheat, the callus must be removed from the test tube culture and placed on another medium to get plants to form."

Getting the cultures to form a callus has proven to be a first and necessary step for researchers. Their best success to date comes from a culture medium consisting of sucrose, plant growth hormones, iron and an extract from boiled potatoes. Although they do not know exactly what it is in potatoes that causes a callus to develop, Margurger and Sammons have found that peeled potatoes produce a 67 percent increase in the number of anthers with callus over potatoes with skins.

"The potato skin apparently contains some retarding substances," says Marburger.

One of the best environmental conditions for callus formation, according to a study by Marburger and Sammons, is to subject anthers to a cold treatment of 4 degrees Centigrade for approximately 1 week.

"Warmer or colder temperatures, or shorter or longer incubation periods provided variable frequencies of callus formation, depending upon the cereal species studied," Sammons says.

Chemical treatments offered some promise of success, according to the two scientists. Barley researchers tried what they call conditioned media. It proved effective in barley, but seemed to inhibit callus formation in wheat, says Sammons.

"Hormone solutions applied to plants before anther culture seemed to have no effect," he adds. "It might prove useful in future research to determine the constituents of the anther culture medium that induces pollen formation in wheat. We should then look more closely at environmental conditions affecting anther culture," Sammons says.

Third Generation Fungicides —
Environmentally Sound Chemicals

In the world of chemical pesticides, emphasis now is on finding ways to protect agriculturally important crops without introducing harmful substances to the environment. A basic understanding of how and why certain chemicals are successful protectors of crops against invading diseases usually precedes any massive assault on the disease itself.

The work of botanist Hugh D. Sisler is a good example. Last year Sisler was named Outstanding Researcher in The University of Maryland's Division of Agricultural and Life Sciences for his studies of the basic mode of action in important fungicides.

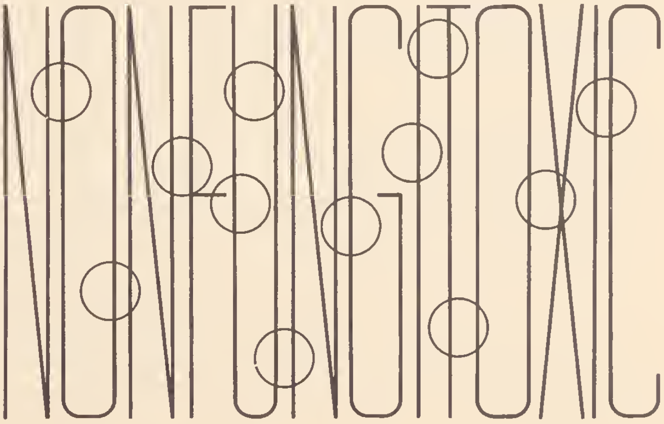
Those fungicides are commonly called **nonfungitoxic** compounds because they do not actually kill an invading fungal pathogen or invading parasite. Instead, says Sisler, they help the host plant bolster its own defense mechanism against attack.

"Some of them actually interfere with the invading parasite's ability to penetrate the plant epidermis. If the fungal pathogen cannot get through the topmost layer of protective cells, the plant has a better chance of fighting off the disease itself," says Sisler.

One important nonfungitoxic compound Sisler studied was tricyclazole, which some botanists believe could be part of a major new generation of environmentally sound chemical treatments for disease in crops. Sisler found that tricyclazole, and several other compounds, prevent pathogens from penetrating the plant epidermis by inhibiting the biosynthesis of sterol compounds in the plant. Sterols are a class of solid complex alcohols.

Sisler says plant scientists need to learn more about these compounds, however, because individually each has a narrow range of disease control. That means that each of these chemicals is capable of treating perhaps only one or a very small number of specific fungal diseases.

One of the nonfungitoxicants' major benefits, however, is that fungi do not build up immunities to them. As Sisler sees it, the new generation of fungicides will help plants erect their own defenses instead of trying to kill an invading parasite.



Diagnosing Disease, Sleuthing for Pesticides in Food

Progress in science is generally characterized by periods of slow growth, punctuated by periodic and quantum leaps of imagination and revolution. Some say research in animal immunology is on the verge of such a revolution as biotechnology increases our basic understanding of the animal immune system.

Hybridoma technology, in particular, has caught the imagination of a number of UMAES researchers. This technology features the combining of important antibody, but short-lived, cells with myeloma cancer cells that are so long lived they are called "immortal". Hybridoma technology ranges across disciplines from animal science to veterinary science to entomology. These scientists are producing and characterizing monoclonal antibodies, which can be applied to a number of agriculturally important problems such as rapid and precise diagnosis of animal viruses, detection of pesticides in food products, and drug residues in milk. These monoclonal antibodies are called "magic bullets" by some scientists because, unlike their less refined cousins the polyclonal antibodies — which occur naturally when disease invades an animal's body — monoclonal antibodies are specific detectors of one and only one invading antigen. They capably ignore every target but the one for which they were manufactured.

The result is a disease detection system that spends precious little time on unimportant antigen cells in an animal's body. Instead, monoclonal antibodies, rapidly and precisely, detect more important diseases coursing through an animal.

Following are some of the major endeavors by UMAES scientists to analyze health problems using biotechnological methods.

ELISA Comes Through on Avian Influenza

Veterinary scientists David B. Snyder and Warren W. Marquardt adapted **ELISA,** the rapid diagnostics system, in mid-1984 to detect avian influenza, a disease responsible for the loss of more than 14 million chickens and turkeys in the mid-Atlantic region in 1983 and 1984.

According to the two researchers, having the system 2 years ago could have saved poultry farmers and allied industries in Pennsylvania — the hardest hit state — at least some of the estimated $150 million lost through the quarantine and destruction of flocks.

"The most often-used method of detection is so insensitive to the disease that it may have lulled the poultry industry into a false sense of security," says Snyder.

ELISA has been used to diagnose other types of poultry viruses, such as infectious bronchitis and Newcastle disease, according to Snyder and Marquardt.

Laboratory and field tests of **ELISA** showed it is more sensitive, diagnoses avian influenza faster, and diagnoses it earlier in the life cycle of the disease than other conventional forms of disease detection. Even when concentrations of natural antibodies to the disease are low in infected flocks, **ELISA** can detect avian influenza. **ELISA** also can analyze hundreds of flock blood samples in a few hours, compared to the 12 to 48 hours needed for conventional tests. The lab and field tests also showed **ELISA** can detect avian influenza as early as 8 days after infection, compared to 21 to 35 days for conventional detection systems.

Not having the sophisticated tools like **ELISA** to detect avian influenza early enough may have played a major role in the final destruction of more than 14 million birds in the mid-Atlantic region, according to most animal disease experts.

Enzyme-Linked Immuno-Sorbent Assays ELISA

Rapid Diagnostics: A Scientist's Munitions Factory for Magic Bullets, Six-Guns and Gunfighters

For 50 years, veterinary and animal scientists have been trying to cope with avian diseases such as infectious bronchitis (IBV), a highly contagious respiratory virus that attacks young broiler and layer chickens. Left unchecked, or discovered too late, a poultry farmer can watch a large part of his flock die before his eyes in a matter of weeks from IBV.

Vaccine production cannot keep pace with the number of new IBV variants, so early diagnosis appears to be the most effective weapon in a poultry producer's arsenal.

In spite of a plethora of new vaccines, outbreaks still occur. Sometimes the vaccines themselves cause disease in "stressed" birds. Sometimes a new form of the virus is "imported" to an area where birds were vaccinated for the "old" form. And, other times, the same old form of the virus mutates, and produces variants for which the old vaccine does not work.

It is for particularly the latter problem that today's virologists are looking for rapid and precise tools to diagnose newly emerging forms of IBV soon enough to save hard-working owners their flock and livelihood.

According to immunologists Dr. David B. Snyder and Dr. Warren W. Marquardt, monoclonal antibodies come closest to the magic bullet science needs to diagnose disease early. Monoclonal antibodies are highly specific proteins, used as markers, or probes. They will attach themselves only to the specific invading substance for which they were manufactured. In doing so, they help scientists quickly identify the variant of a virus with which they are dealing.

"Monoclonal antibodies help us define the antigenic repertoire of IBV," says Snyder. "That is important to the poultry industry, because it helps prevent disease. It is important to virologists like us because we want to know if new types of the virus are evolving."

So far Snyder and Marquardt have developed a battery of monoclonal antibodies to diagnose nearly all of the known serotypes and strains of IBV.

In terms of time, why are monoclonal antibodies so important? Before science knew much about them, it took months for researchers using traditional laboratory methods to isolate and identify the disease-causing antigen in a flock of chickens. By that time, it was too late to help a producer.

Monoclonal antibodies ushered in an era of **rapid diagnostics.** Today, scientists like Snyder and Marquardt can identify a serotype of IBV in about 3 hours.

They have coupled the production of monoclonal antibodies with another research tool to speed up the process even more. **ELISA** (Enzyme-Linked ImmunoSorbent Assays) is a new computer-linked test that measures the level of antibodies in the blood of animals and poultry. It is, essentially, the six-gun that loads and fires monoclonal antibody "magic bullets".

"Our method is fully automated," says Snyder. "It accomodates up to 10 times the number of test samples as conventional tests at approximately 20 percent of conventional

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Fluorescent Dyes and Rabbits

Tools of Precision and Convenience to Build Tomorrow's Super Cow

Embryo transfer has become an almost commonplace procedure in animal husbandry. It can produce a lot of quality offspring from one set of genetically superior parents.

In cattle, for example, the procedure starts with a fertility drug called **FSH**, follicle stimulating hormone. Given to a cow, it causes her to "superovulate", release several ova rather than the usual one. Breed a champion cow who has superovulated to a prize-winning bull, and chances are you will wind up with several embryos just waiting to mature into champions themselves.

Carrying multiple offspring to full term is asking a lot of any mother. That is where the actual "transfer" process in embryo transfer comes in. Skilled hands flush the donor mother's uterus with a phosphate buffered saline solution, washing the solution and the developing embryos into a flask. Using a microscope, technicians sort out the individual embryos and transplant each into a surrogate mother who will carry the fetus to term.

If you are thinking by now there are a lot of places along the way for something to go wrong, you are right. Finding ways to keep things from going wrong keeps veterinary researcher Dr. Monica Haaland busy.

"One important consideration is determining what we call the embryo's viability before we go to the trouble and cost to transfer," says Haaland, an assistant professor in the Maryland-Virginia Regional College of Veterinary Medicine.

"In other words, what are its chances of survival in the surrogate mother? For embryos of questionable quality, in particular, currently we have to place the embryo in culture for 12 to 24 hours and look for progression to a more advanced stage of development," says Haaland.

That, she points out, is very time consuming and costly because the process has to be done individually for each embryo.

Another real-life related problem is, what do you do if the donor mother is in Maryland, but the surrogate mothers are scattered throughout the world?

For years, commercial firms have frozen embryos in liquid nitrogen, much the same as vats of liquid nitrogen have kept the sperm of prize-winning bulls, long since departed from this Earth, intact and ready to fertilize generations to come. The dependability of thawed embryos, however, is not on par with that of thawed bull semen. The pregnancy rate for thawed embryos, for example, is only about 50 percent that of fresh embryos.

"In both cases of fresh and frozen embryos, we need a rapid and conclusive tool to evaluate their viability before they are transferred to a surrogate mother," says Haaland.

That tool may be the fluorescent dye technique Haaland is working with now. The dye works as a marker, lighting up viable embryos like tiny fluorescent lights under a microscope. Whether they are fresh or thawed embryos, the ones that stand the best chance of survival later in a surrogate mother can then be separated from those that have little chance of survival, according to Haaland.

Although the process has been around since at least 1979 on an experimental basis, it is not foolproof.

"Some dyes work better as markers than others. With some, there is a strong relationship between the dye's ability to mark an embryo and that embryo's ability to continue to undergo development. And we cannot exclude the toxic or nontoxic effects dyes may have on the developing embryo," says Haaland. "The more we know about the dye technique, the better our chances of improving transfer techniques and increasing a surrogate mother's chances of carrying a calf to full term."

Cattle Make Way for Rabbits in Superovulation Studies

Haaland's studies of improving embryo transfer techniques in cattle moved along slowly until she decided to stop using cattle embryos and concentrate instead on rabbit embryos.

Why? A cow given FSH fertility drugs will superovulate an average 5 to 10 ova at most. Rabbits, on the other hand, do just what rabbits are famous for doing — they will superovulate 20 or more ova at one time.

(continued on last page)

Monoclonal Antibodies:

They Aren't Used Just to Recognize Disease

Do you know what is in the food you eat, and what effect it might have on your health? For some substances, scientists are not even sure how much of a particular item is considered harmful.

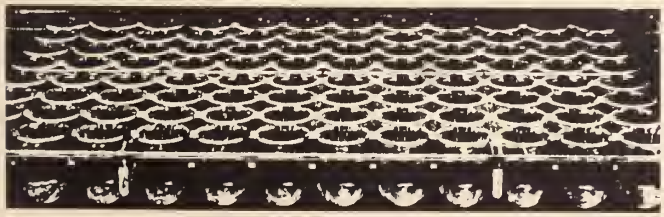
Take for example the chemical **maleic hydrazide,** MH, a pesticide used as a plant growth inhibitor in agriculture, primarily for tobacco, but also for some food products such as potatoes and onions. MH prevents sprouting during storage of these food products and it appears to be harmless to humans.

The ensuing debate in the scientific community over how much MH can be found in food products, whether MH is harmful to humans, and how much of it you would have to eat before it became harmful, is complicated by one major problem. It is extremely tedious to detect MH residues in plant or animal tissue. Conventional methods, such as multiple residue analysis, simply do not pick them up.

Entomologist Dr. Judd O. Nelson, a university colleague, and researchers from the Uniformed Services University of Health Sciences (USUHS) wrestled with the problem of how much MH can be found in food products. They are turning to the immunologist's newfound friend — **monoclonal antibodies.** Monoclonal antibodies are traditionally thought of as "magic bullets," capable of recognizing specific disease antigens. They are a rapid and precise aid in diagnosing diseases in animals and humans. According to Nelson, more and more researchers are turning to **immunoassay** — the process of producing monoclonal antibodies and using them to scan for specific substances — to detect pesticides such as MH.

"Potentially, the technique is easier, less expensive and more versatile than currently used methods of analysis," Nelson says.

The group is confident that, indeed, it may be possible to produce monoclonal antibodies that bind to several forms of MH. These antibodies are capable of recognizing the difference between MH and other substances. This is based on their recent work with polyclonal antibodies, which are less refined versions of monoclonal antibodies. That, says Nelson, is a basis for developing sophisticated scanning technology for levels of pesticides such as MH in foods.

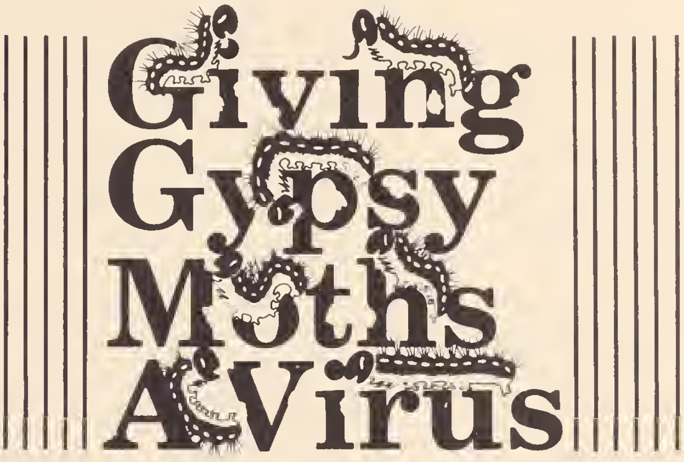


What used to be the golden sounds of silence in this country's forests in the Northeast have given way to the sounds of breakfast, lunch and dinner. Gypsy moths are munching their way south, defoliating whole forests in New England and the mid-Atlantic states.

Maryland entomologists are trying to find a way to turn the tables. They are seeking a variant of wilt disease that is unique to gypsy moths and, ultimately, kills them.

What appears at first glance to be a simple matter of sleuthing for the right virus is anything but, according to Dr. Michael C. Ma, assistant professor of entomology. Left to its natural course, says Ma, **nuclear polyhedrosis virus** (NPV) affects gypsy moths only when their numbers have grown large enough to eat themselves out of house and home. Hungry and overcrowded, they are most susceptible to the disease.

"But by that time, they've already defoliated an area," says Ma.



Giving Gypsy Moths A Virus

Is there a way to make the moths more susceptible before a forest is bare, then initiate an epidemic of NPV and watch the moths' numbers dwindle?

That is one of the major questions Ma has been studying for several years.

"We know too little about the life cycle of the virus in its natural habitat, the forests, to come up with some strategy to infect whole populations of gypsy moths," says Ma.

One way to determine the presence and amount of a virus in a forest is through the use of immunological methods, according to Ma. Monoclonal antibodies are very specific proteins, cloned in a laboratory to produce a large number of identical markers or probes that will determine, with pinpoint accuracy, the presence of a specific antigen or virus.

Ma has developed a low-cost test that can tell a forester if the virus is present in a gypsy moth population, and how much of the virus is there — all in a matter of hours. The test can help researchers learn more about the life cycle of the virus in the wild, and thereby find some way to use the virus more effectively as a natural control agent.

The U.S. Environmental Protection Agency (EPA) approved the use of NPV as a biological insecticide in 1978 and the U.S. Forest Service adopted Ma's rapid diagnostic system in 1984.

(continued on last page)

HIGHLIGHTS '84

The following are brief excerpts of other accomplishments of The University of Maryland Agricultural Experiment Station in 1984.

In conjunction with the state of Maryland's efforts to clean up the Chesapeake Bay, UMAES cosponsored educational symposia during the year to focus attention on agriculture's role in the cleanup. UMAES scientists are helping inform farmers about nonpoint source pollution — primarily from farms and forests — and its damaging effects on bay aquatic life.

Also in conjunction with the Chesapeake Bay cleanup effort, UMAES director W. Lamar Harris and other UMAES personnel are key participants in the Technical Team for Agricultural Water Quality, appointed by the State Soil Conservation Committee. The team is developing recommendations for "best management practices" for farmers to use to stem the tide of agricultural pollution entering the Bay.

Since the mid-1950's, UMAES has supported the collection of meteorological data for the state of Maryland. Now, these records — some of which date back 75 years — are readily available to agricultural scientists, Extension agents and pest management specialists to help them do their jobs better. The computer-stored data are especially helpful to researchers who may wish to use them for statistical summaries and analysis in their research.

In mid-summer 1983, the university broke ground at its Eastern Shore campus (UMES) for a new Poultry Research and Education Facility. Now completed, this facility will support the poultry research programs of UMAES, the resident instruction needs of UMES, and the outreach programs of the university's Cooperative Extension Service — all in the heart of Maryland's flourishing poultry industry. The $625,000 project includes four major buildings to house laboratories, offices, controlled environment studies and an experimental production house.

In early 1984, UMAES broke ground for another research facility, at the university's Tobacco Experimental Farm near Upper Marlboro. This facility, too, is now complete and is called The Southern Maryland Research and Education Facility. Studies there will focus on tobacco variety improvement and harvest mechanization, agronomy, agricultural engineering, botany and horticulture research. The $178,000 facility includes a 2,100-square-foot building that will house laboratories and offices.

Maryland's Research Farms

The University of Maryland College Park
UMAES Headquarters (301) 454-3707
Research work in all phases of agriculture and related fields.

Plant Research Farm (Montgomery county)
Research on insects, truck crops and small fruit. 194 acres. (301) 572-7247 — Agronomy; (301) 572-5339 — Horticulture.
Turfgrass Research and Education Facility (Montgomery county)
Research on turfgrass. 37 acres.

Agronomy — Dairy Forage Swine Research Farm (Howard county)
Studies of dairy nutrition and management and pollution abatement practices. 922 acres. (301) 596-9550 — Dairy Forage; 531-2553 — Swine Research Unit.

Horse Research Farm (Howard county)
Research on physiology, nutrition and management of horses. 154 acres. (301) 465-3760.

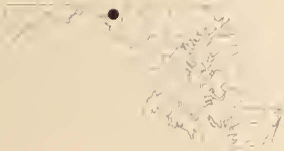
Beef Research Farm (Carroll county)
Research concerning livestock production and management. 715 acres. (301) 795-1310.

Southern Maryland Research and Education Facility (Prince George's county)
Research relating to tobacco breeding, production, harvesting and curing. 206 acres. (301) 627-3273.

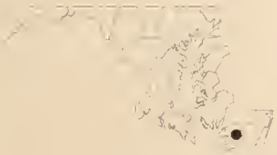
Wye Research and Education Center (Queen Anne's county)
Work on plant breeding, weed and disease control, and production systems for corn, soybeans, vegetables and ornamentals. 123 acres. Additional research in cooperation with Wye Institute. 355 acres. Beef cattle research with Wye Angus herd. Approximately 475 acres on Wye Plantation. (301) 827-6202 — Center Headquarters.

Salisbury Research Substation (Wicomico county)
Experimental studies dealing with poultry and breeding; insect, pest and disease control; production systems; and management and processing of vegetable crops. 125 acres. (301) 742-8788 — Horticulture; (301) 543-6700 — Poultry Science.

Poplar Hill Research Farm (Wicomico county)
Studies of disease control, breeding, pest control and production systems for corn, soybeans and vegetable crops. 166 acres. (301) 742-9694.



Western Maryland Research and Education Center (Washington county)
Research on fruits, vegetables, ornamentals, field crops, soils, and disease and insect control. 546 acres. (301) 791-2298.



The University of Maryland Eastern Shore
UMAES 1890 Agricultural Research Program
Research work in poultry, human nutrition, pest control and cultural practices for soybeans and corn, small farm development and child development. (301) 651-1598.

Biomembranes

In spite of a less-than-dramatic role in crop improvement now, protoplast fusion will be a credible technique for genetic manipulation in the future, Bottino asserts. Its eventual products — somatic hybrid plants — will have greater genetic variability than comparable sexual hybrids.

Protoplast fusion also will serve as a starting point for new breeding techniques once somatic hybrids are available.

"The idea would be to select somatic hybrids showing variation for a particular trait of value, and backcross them into the cultivated parent," Bottino says. "This combines gene transfer with conventional breeding techniques."

Protoplast fusion may be used as a tool to manufacture **bridge hybrids.** These hybrids can help breeders overcome sexual incompatibilities between two traditional plant species by providing a somatic hybrid and genetic link between the two.

Perhaps the most valuable use of protoplast fusion lies in transferring cytoplasmically controlled traits, according to Bottino.

"When protoplasts are fused, regardless of what happens to their nuclei, the organelles of the two cells mix together in the common cytoplasm," he says. "This produces a cell hybrid — or **cybrid** — containing a mixture of organelles from the two sources."

Cell division produces a random partitioning of organelles which, ultimately, can result in new combinations of the two genetic systems that were never dreamed possible.

Fluorescent Dyes and Rabbits

"Once they're fertilized and flushed from the uterus of the rabbit, that gives us more embryos to study and manipulate at one time. And that saves us time and money," says Haaland.

Rabbit embryos also are useful because they are larger than cattle embryos and easier to manipulate physically. The gestation time for rabbits (37 days) is much shorter than that for cows (284 days). Haaland is working with a technique called **micromanipulation.**

With this technique, a technician splits an 8-day-old embryo in half and inserts each half in an "emptied" donor ovum. There, each half continues development, and each half retains identical genetic characteristics. The result is identical twin offspring delivered by separate surrogate mothers.

"As the technique improves, it should be possible to correct genetic errors by injecting the appropriate DNA material into the embryonic nucleus," Haaland says.

The same techniques applied to rabbit embryos in the preliminary study can be applied more effectively later to cattle, according to Haaland.

ELISA: Rapid Diagnostics

costs. And it takes less than 10 percent of the time required to perform the standard battery of tests for similar viral agents."

Marquardt adds, "The system provides information that was essentially unobtainable before, and at a fraction of the cost. It also provides that information in time for flock owners to make critical management decisions."

If monoclonal antibodies have found a suitable six-gun in the ELISA system of rapid diagnostics, the two together have found a worthy aiming device in the form of a management tool called **flock profiling.**

According to veterinary scientist Dr. Edward T. Mallinson (who works with Snyder and Marquardt), flock profiling is a method of long-term health monitoring come of age, thanks to the technological advances of monoclonal antibodies and ELISA.

"Flock profiling provides a way to monitor continuously the health of a flock for its entire life," says Mallinson.

Because chickens cannot tell you when they are sick, the best method to detect these deadly infections is to measure the levels of antibodies in a flock periodically, to pick up signals that an immunity is waning or an infection is rising. ELISA can pick up those signals in a very short time.

"The key to flock profiling is to spot trends early enough so that an owner can prevent a serious problem," says Mallinson.

The time factor is especially crucial for the Delmarva broiler industry where broilers go to market only 7 weeks after hatching.

Gypsy Moths

"The virus itself is safe to virtually everything but the gypsy moth," says Ma. "It does not infect mammals or humans, or other insects or animals that are natural enemies of the gypsy moth."

A major stumbling block to the use of the virus as a control agent is the typical one for most viruses: Every known virus has variants. So the problem for researchers using monoclonal antibodies as a diagnostic tool is to come up with specific tagging antibodies for each and every one of those variants.

"Monoclonal antibodies which are specific to different components of the virus might help us study how the virus causes disease in an insect pest like the gypsy moth," Ma says.

DO NOT CIRCULATE

bi·ol·o·gism /bi- äl-ə-jiz-əm/ *n* : preoccupa- tion with biological explanations in the analysis of social situations - **bi·ol·o·gis·ti** /- äl-ə- jis-tik/ *adj*

bi·ol·o·gy /bī- äl-ə-je/ *n* [G *biologie*, fr. *b* + *-logie* -logy] **1 a** : a branch of knowledg that deals with living organisms and vita processes **b** : ECOLOGY **2 a** : the plant an animal life of a region or environment **b** the laws and phenomena relating to an organism or group — **bi·ol·o·gist** /-jəst/ *n*

bio·mass / bī-ō-mas/ *n* : the amount of liv ing matter (as in a unit area or volume c habitat)

bio·med·i·cal /bī-ō- med-i-kəl/ *adj* **1 :** of o relating to biomedicine **2 :** of, relating to, or involving biological, medical, and physical science

bio·med·i·cine /med-ə-sən, *Brit usu* - med sən/ *n* : a branch of medical science con cerned esp. with the capacity of huma [illegible] ve and function in abnorm [illegible] ts and with the protectiv modification of such environments

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